TABLE II.
Influence of Salt upon Bacterial Viability and Diffusion of Ammonia into the Menstruum in Terms of mg. per Cell per Hour Expressed as Percentages of Results in Salt-free Control.

	M NaCl	Exp. 1	2	3	4	5	Average
Final	0.05-0.08	133	111	90	103	112	110
Bacterial	0.8-1.0	97	77	61	83	35	71
Count	2.0	57	70	63	85	72	69
Diffused	0.05-0.08	137	125	141	103	130	127
Ammonia per	0.8-1.0	89	92	73	53	113	84
Cell per Hour	2.0	68	67	46	62	77	64

outward through the cell wall. The short time period employed, in the absence of nutrient materials, makes it reasonably clear that changes in production of ammonia within the cell are not the determining factors. We may conclude then, that a dilute stimulating concentration of NaCl increases and a strong toxic concentration decreases diffusion of metabolic products outward through the cell wall. Whether this is due to an influence on the permeability of the cell membrane or to an effect on physico-chemical conditions within or without the cell is uncertain.

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Cellular Responses to Acetone-Soluble Lipoids from Mycobacteria.

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The cellular responses to phosphatides and the so-called waxes isolated by Anderson[1] from acid-fast bacilli have been studied by Sabin and Doan,[2] Sabin, Doan and Forkner,[3] and by Smithburn and Sabin.[4] Anderson[1] has separated from the bacilli a third lipoidal component which is soluble in acetone and which is recovered as a soft brown, salve-like solid with an odor not unlike that which arises from cultures of tubercle bacilli. This substance was shown[1]

[1] Anderson, R. J., *Physiol. Rev.*, 1932, **12**, 166.

[2] Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, **46**, 645.

[3] Sabin, F. R., Doan, C. A., and Forkner, C. E., *J. Exp. Med.*, 1930, **52**, Suppl. No. 3, 3.

[4] Smithburn, K. C., and Sabin, F. R., *J. Exp. Med.*, 1932, **56**, 867.

to consist largely of a mixture of fatty acids. The cellular responses induced by this acetone-soluble lipoid will be discussed in the present communication.

Rabbits were used in the experiments. The lipoids employed were isolated from human, bovine, and avian tubercle bacilli, and the timothy grass bacillus and were injected intraperitoneally. The acetone-soluble lipoid is not miscible with water and was therefore suspended in mineral oil for administration in the first experiments. Later it was made neutral to litmus with N/10 NaOH and suspended in water for injection. The material was also given undiluted. Cellular studies were made of the fresh tissues by the supravital and by the fixed tissue methods.

The acetone-soluble fat was found to be the most irritating of the crude lipoids from acid-fast bacteria. It induced extreme vascular dilatation, proliferation, and hemorrhages. It is the only substance from tubercle bacilli yet tested in this laboratory which has produced massive adhesions. Perhaps due to the content of phthioic acid,[1, 3] it induced the formation of epithelioid cells in considerable numbers. In addition, this lipoid caused marked stimulation and proliferation of every type of connective tissue cell and it always called forth leucocytes in large numbers. Many of the leucocytes were contained in clasmatocytes; others were free. Red blood cells and droplets of mineral oil were also seen within clasmatocytes. Many undifferentiated connective tissue cells, fibroblasts, lymphocytes, and plasma cells also characterized the reactions. No particular type of cell was dominant in the reaction. These changes were noted after administration of the lipoid undiluted and also when it was suspended in mineral oil.

In subsequent experiments the acetone-soluble fat was made neutral to litmus with N/10 NaOH and suspended in water for injection. This procedure brought about a marked reduction in the irritating properties of the lipoid. After administration of the neutralized material, only a minimal number of epithelioid cells appeared in the tissues. There was also a diminution in the proliferation of other types of cells, as well as in the vascular changes. However, neutralization did not reduce the power of the lipoid to induce the formation of adhesions which have been found to persist for at least one month. In this connection it is interesting to note that, whereas the phosphatide and fatty acid I (phthioic acid) from tubercle bacilli produced epithelioid cells in great numbers,[3] this property was lost when the substances were neutralized with KOH.[5]

[5] Sabin, F. R., and Smithburn, K. C., unpublished data.

Unquestionably some of the irritating properties of the tuberculolipoids are largely due to the presence of free fatty acids.

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Tetanus Toxoid in Prophylaxis against Tetanus.

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Ramon[1, 2, 3] and his colleagues have applied their method of detoxifying diphtheria toxin with formaldehyde to the detoxification of tetanus toxin. The addition of 0.3 to 0.4% of commercial formaldehyde to tetanus toxin causes detoxification at incubator temperature in about 2 weeks. A tetanus toxin containing 5,000 to 10,000 MLD per cc., for guinea pigs, when detoxified can be injected subcutaneously into guinea pigs weighing 300 to 350 gm., in doses of 5 cc. without giving rise to symptoms of intoxication.

Ramon and his colleagues state that 3 doses of 1 cc. of toxoid injected subcutaneously into guinea pigs at 14 day intervals induce a high degree of immunity.

The purpose of this study was to ascertain the protective power and immunity afforded to guinea pigs by 3 doses of tetanus toxoid. The degree of protection obtained was determined by measuring the neutralizing power of the serum of the immunized guinea pigs when administered to normal guinea pigs combined with 0.1 L+ dose of standard tetanus toxin.

We studied the immunizing effects of 2 toxoids, one No. 6 prepared from a toxin containing 3,000 MLD per cc., and the other, prepared from a toxin containing 10,000 MLD per cc. Titration of the sera of 3 guinea pigs immunized with toxoid No. 6 indicated the presence of 0.2 of a unit of antitoxin per cc. of blood-serum, while titration of the sera of 5 guinea pigs immunized with toxoid No. 7 indicated the presence of 0.5 of a unit of antitoxin per cc. of blood serum.

On the basis of the titrations of the antitoxic content of the sera of the guinea pigs treated with toxoids No. 6 and No. 7, the animals received the following doses of tetanus toxin:

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[1] Ramon, G., *Airn. d., Inst. Pasteur.*, 1924, **38**; *C. R. Acad. Sci.*, 1924, **78**, 436.

[2] Descombey, F., *C. R. Soc. Biol.*, 1924, **91**, 239.

[3] Zoeller, Chr., and Ramon, G., *Presse Med.*, 1926, **164**, 485.